PRELIMINARY DATA COMUNICATION

"Placebo-controlled assessment of the efficacy of a food supplement in decreasing the UVB-induced skin redness and the skin ageing signs"

Customer: **Monteloeder**
Tested product: **Nutroxsun**

San Martino Siccomario, May 20th, 2015

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1. STUDY DESIGN

1.1. Aim

The study was aimed to assess the efficacy of the food supplement NutroxSun™ in decreasing the UVB-induced skin redness and the skin ageing signs.

1.2. Tested product

1.2.1. Information provided by the Customer

☒ Product name: **Nutroxsun**
☒ The product to be tested is a 50:50% mixture of *Citrus paradisi* and *Rosmarinus officinalis* extract
☒ How to use: 1 capsule per day at breakfast

1.3. Subjects

Eligible subjects were all adult female subjects having skin phototype from I to III according to Fitzpatrick classification. Subjects were enrolled by a dermatologist according to the inclusion/non-inclusion criteria reported here below.

1.3.1. Inclusion criteria

✓ Good general health
✓ Skin type from I to III according Fitzpatrick classification[1]
✓ Clinical signs of chrono- or photoaging[2]
✓ Caucasian skin type
✓ Age older than 18 years old
✓ Adequate rest period between two similar study
✓ Willingness to not use products likely to interfere with the product to be tested

[1] from I to III for the long-term test (n = 90, 30 subjects per group) and II to III for the short-term test (n= 5)
[2] inclusion criteria relevant for the long-term test

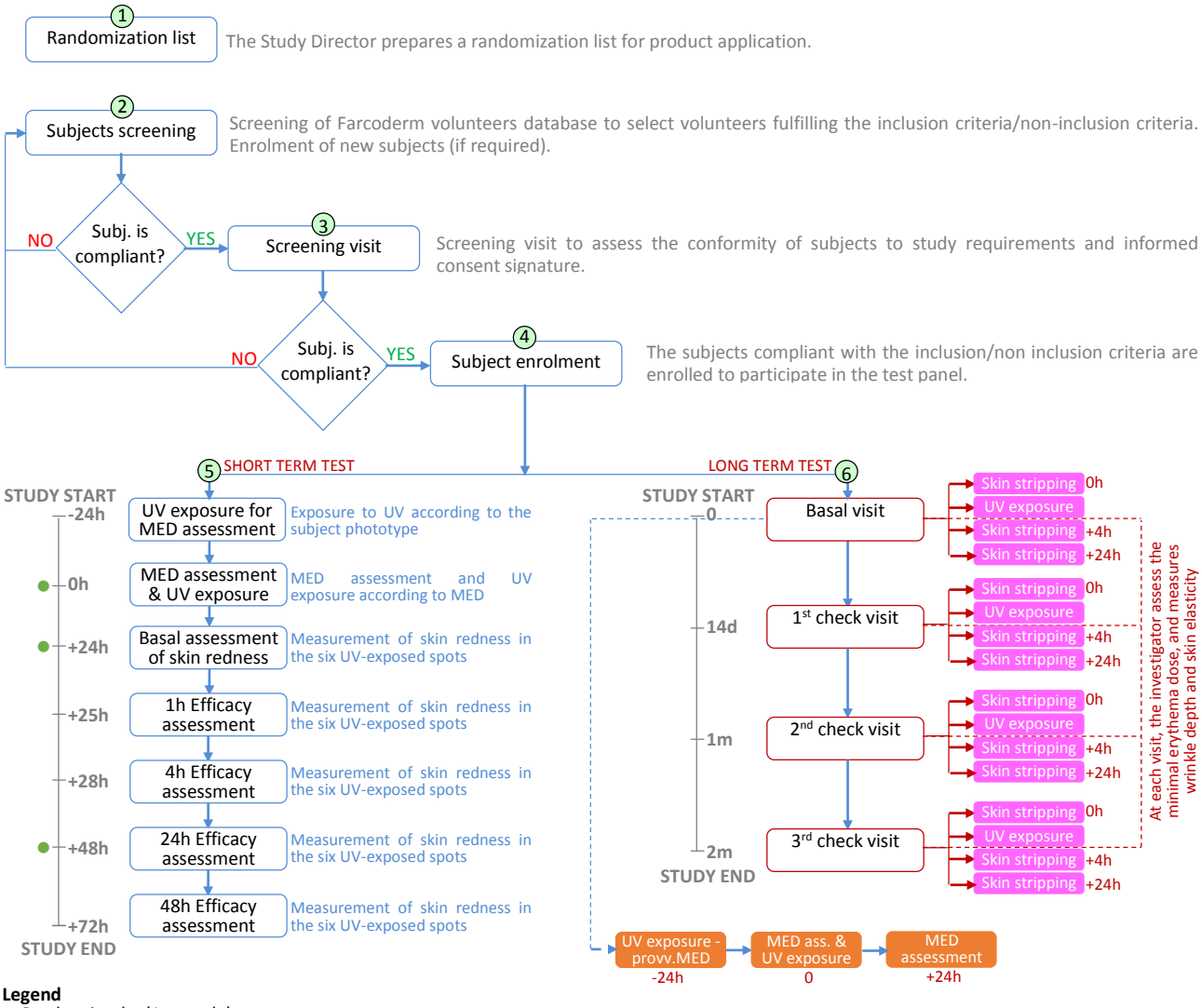
✓ Willingness to not use, during all the study period, face creams other than the products supplied
✓ Test area (back) is uniform in colour, without nevi, blemishes or solar lentigo and without hairs
✓ Subjects who have not sun exposure on the back area for at least two months prior to the study
✓ Willingness to not vary the normal daily routine
✓ Subject is under effective contraception (oral/not oral); not expected to be changed during the study

1.3.2. Non-inclusion criteria

- Subject do not meet the inclusion criteria above reported
- Alimentary/Eating disorders (i.e. bulimia, psychogenic eating disorders, etc.)
- Food allergy or food intolerances
- Sunburn, suntan, scars, or active dermal lesions on the areas of the back selected for the test purposes
- Subjects having used self-tanning products on the back in the previous month after the date of the study
- Subjects accustomed to using tanning beds
- Subjects taking medication with photosensitizing potential, drugs and/or dietary supplements able to induce skin colouring, corticoids, currently or during the month before the study
- Subjects taking anti-histaminic or anti-inflammatory drugs, currently or within the week before the study
- Impaired immune system due to immunosuppressive diseases such as AIDS and HIV, or use of immunosuppressive medications
- Pharmacological treatment (topic or systemic) know to interfere with the tested product or having effect on metabolism
- Severe concurrent diseases
- Any condition that the principal investigator deems inappropriate for participation
- Pregnant or breastfeeding women
- Adult protected by the law (under guardianship, or hospitalized in a public or private institution, for a reason other than the research, or incarcerated).
- Volunteer unable to communicate or cooperate with the Investigator due to language problems, poor mental development, or impaired cerebral function.

1.4. STUDY DEVELOPMENT

The study duration is 5 days for the short-term test study and 2 months for the long-term test study. The figure here below reports the study flow charts.



Legend
● Product intake (1 capsule)

1.5. MATERIALS AND METHODS

1.5.1. Treatment(s)

The product to be tested is a 50:50% mixture of *Citrus paradise* and *Rosmarinus officinalis* extract. The Placebo product is a 100% maltodextrin capsule.

1.5.2. Provisional Minimal Erythema Dose and Main Minimal Erythema Dose (MED)

Before starting the main test, a provisional individual MED is determined in order to centre the UV dose ranges for the exposure during the main test. This is performed by applying a preliminary series of UV exposures the day before the main test. MED is determined by applying an incremental progression of UV doses based on subject phototype.

1.5.3. Skin redness (only in the short term test, measured on the back)

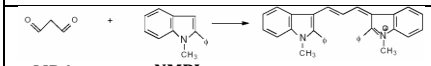
The skin erythema reaction after a series of UV exposures (6 exposed sites using a series of UV exposures based on MED) is measured using a colorimeter/spectrophotometer CM-700D (Konica Minolta). The parameter

measured is the a* parameter of the CIELAB (1976) chromatic space. The a* parameter correlates with skin redness.

1.5.4. *in situ* assessment of the protective effect of the product against UV-induced lipoperoxidation (only in the long term test, measured on the back)

Lipid peroxides are determined on the 10 skin layer**. Malonyldialdheyde (MDA) and 4-hydroxynonenal are the two main products of lipid peroxidation. Their concentration in a biological system is a good index of its lipo-peroxide damage. The level of peroxidation is evaluated by means of MDA test. For further information, see box 1. MDA is assessed before and after 4 and 24h hours from UVA exposure.

Box 1. MDA measurement



MDA **NMPI**

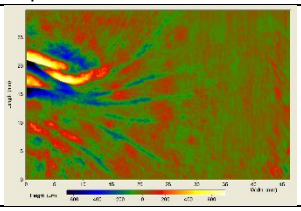
To determine the lipo-peroxides levels it is used the method by Erdelmeier and collaborators (1998). the assay is based on the capability of a cromogen, N methyl 2 phenylindole (NMPI), to react with MDA at 45°C and acid pH to produce a stable chromophore that has an absorption peack at 586 nm. The lipo-peroxide levels are measured after the induction of unstable hydroperoxides decomposition, produced in the oxidative processes by means of a pro-oxidant agent ($CuSO_4$ 500 µM).

** Skin stripping is performed using Corneofix® (Courage+Khazaka). This technique allows to take serial layers of the stratum corneum. In accordance with the standard operative procedure, skin stripping is performed using a device that allows to standardize the pressure applied on the stripping. The first stripping is discarded while strip 10 is collected.

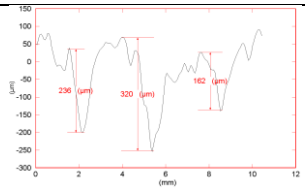
1.5.5. Wrinkle depth (only in the long term test, measured on the crows' feet area)

Wrinkle depth is quantitatively measured using Primos 3D (GFM Messtechnik GmbH). Primos 3D is a non-contact in vivo skin measurement device based on structured light projection. In conjunction with a comprehensive 3D measurement and evaluation software, the sensor allows to evaluate skin surface properties (i.e. wrinkle depth, volume, roughness etc.). For further information, see box 2.

Box 2. Wrinkle depth measurement.



3 dimensional image.



Profilometrical informations of the 3D image.

1.5.6. Skin elasticity (only in the long term test, measured on the cheekbone area)

The measurement of skin elasticity is based on the suction method using a negative pressure deforming the skin mechanically (Cutometer® method). A Negative pressure is created in the device and the skin is drawn into the aperture of the probe for 3 seconds and after a defined time released again. Inside the probe, the penetration depth is determined by a non-contact optical measuring system. The following parameters are measured:

- ✓ R2, (Ua/Uf, gross elasticity or overall elasticity) represent the ability of redeformation of the skin to its basal state.
- ✓ R5, (Ur/Ue, net elasticity) represent the elastic recovery of the skin to its basal state due to its elastic component after deformation. The parameter decays with ageing and it is independent of the skin thickness.

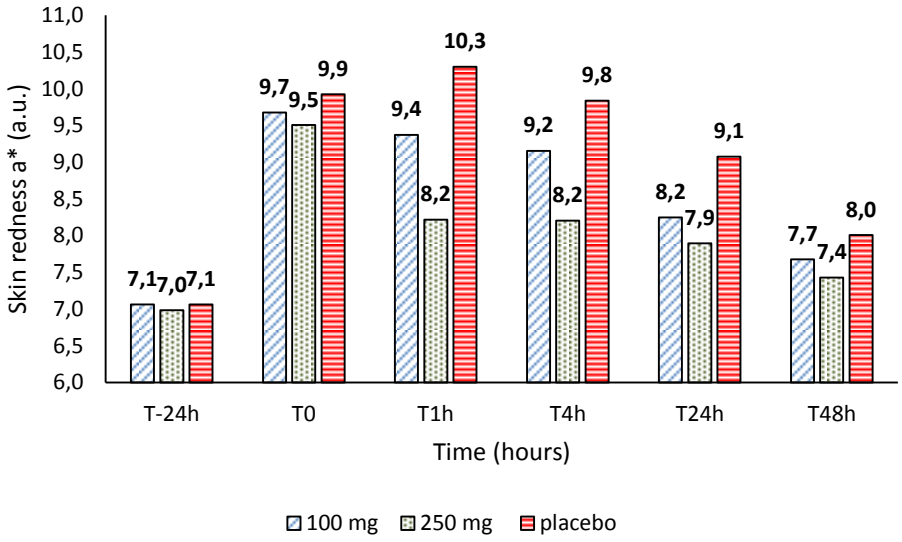
2A. RESULTS SHORT TERM TEST

The effect of the product(s) on UVB-induced skin redness is reported in table 1 and in graph 1. Data (mean value) are reported in arbitrary units (a.u.).

Table 1a. Product(s) effect on UV-induced skin redness (a* parameter).

Intake	✔	✔			✔	
	T0h	T24h	T25h	T28h	T48h	T72h
100 mg	7.1	9.7	9.4	9.2	8.2	7.7
250 mg	7.0	9.5	8.2	8.2	7.9	7.4
Placebo	7.1	9.9	10.3	9.8	9.1	8.0

Graph 1a. Variation of skin redness after product(s) intake.



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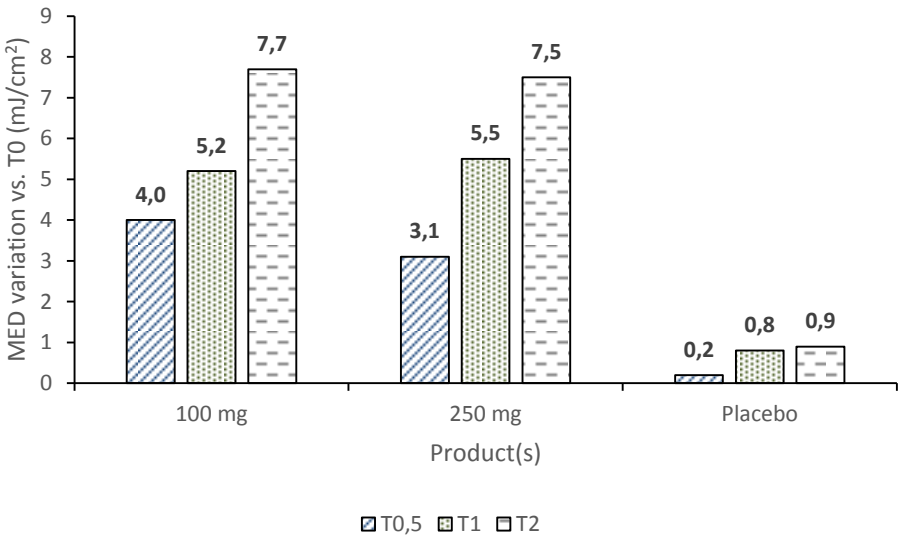
2B. RESULTS LONG TERM TEST

The effect of the product(s) on the measured parameters is reported in table 1 and in the respective graphs.

Table 1b. Product(s) effect on the measured parameters.

	100 mg NUTROXSUN			250 mg NUTROXSUN			PLACEBO		
	0.5M	**1M**	**2M**	**0.5M**	**1M**	**2M**	**0.5M**	**1M**	**2M**
Minimal Erythemal Dose (mJ/cm²)*	+4.0	+5.2	+7.7	+3.1	+5.5	+7.5	+0.2	+0.8	+0.9
Minimal Erythemal Dose**	+15.2%	+20.5%	+29.8%	+11.7%	+20.2%	+26.9%	+1.2%	+2.7%	+3.8%
Skin elasticity - R2**	+1.8%	+3.2%	+4.6%	+1.5%	+2.9%	+3.7%	-0.3%	+0.5%	+0.2%
Skin elasticity - R5**	+3.3%	+5.8%	+9.0%	+2.9%	+5.5%	+7.4%	-0.1%	+0.8%	-0.5%
Wrinkle depth**	-8.8%	-13.4%	-14.8%	-9.1%	-12.6%	-13.9%	+1.2%	-2.2%	+0.8%
LPO - 4h**	-9.7%	-16.2%	-20.1%	-10.2%	-16.4%	-21.7%	+5.0%	+5.4%	+9.3%
LPO - 24h**	-8.7%	-13.4%	-15.1%	-9.1%	-13.3%	-15.8%	+4.5%	+4.4%	+3.8%
Legend * variation vs. T0 (i.e. T_x-T_0). ** % variation vs. T0 (i.e. $[T_x$-$T_0]/T_0$). i.p. in progress									

Graph 1b. Variation of MED after product(s) intake.



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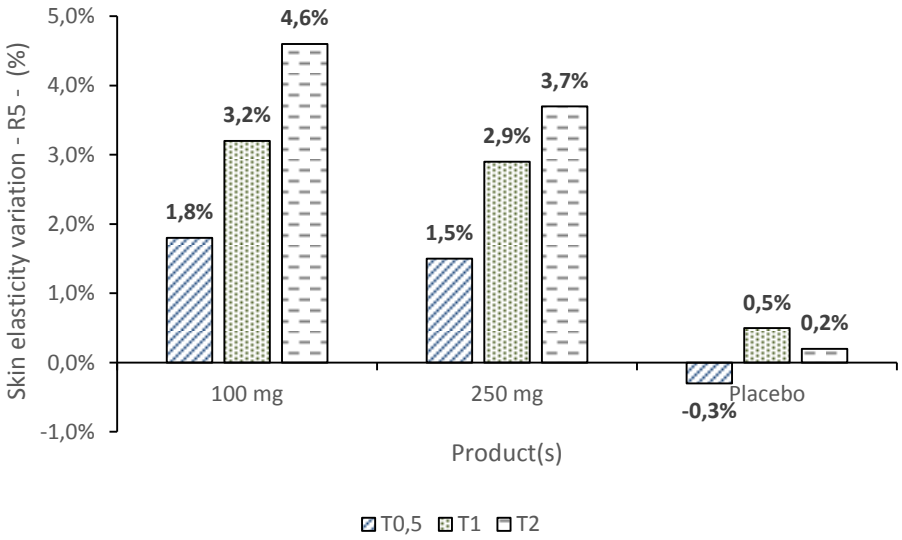
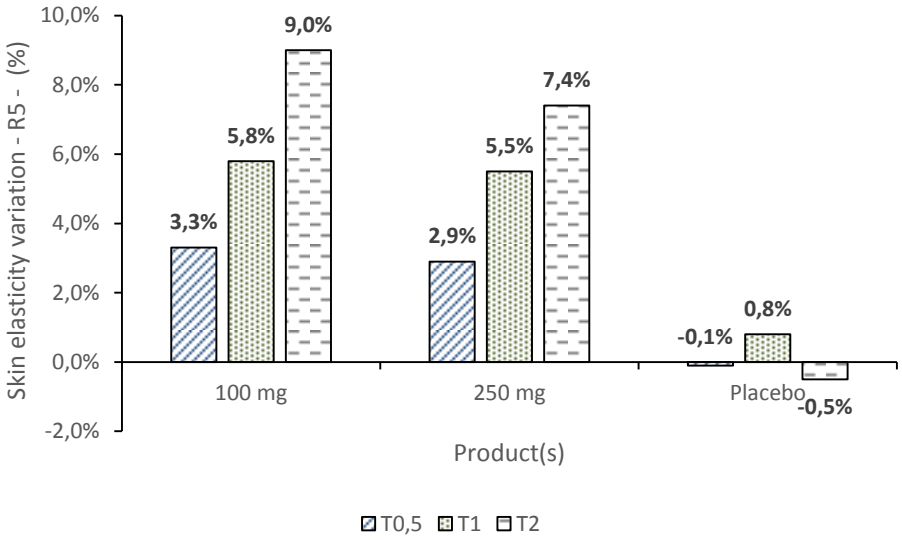
Graph 2b. Variation of Skin elasticity (R2 parameter) after product(s) intake.



Graph 3b. Variation of Skin elasticity (R5 parameter) after product(s) intake.



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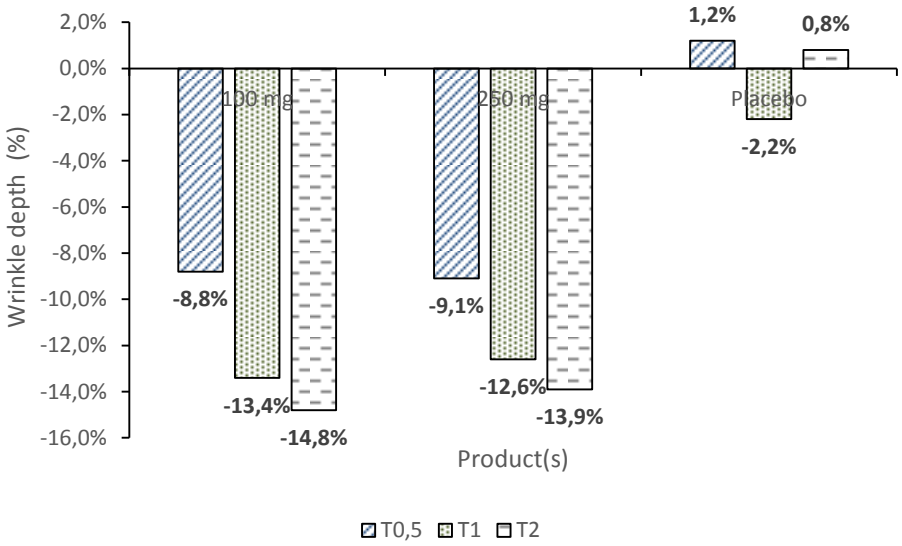
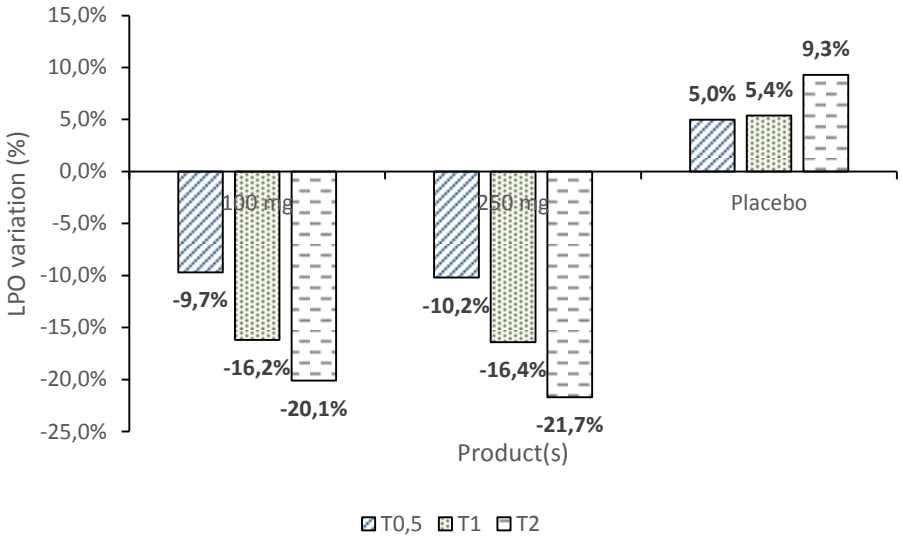
Graph 4b. Variation of wrinkle depth after product(s) intake.



Graph 5b. Variation of UV-induced LPO (4 hours after UV exposure) after product(s) intake.



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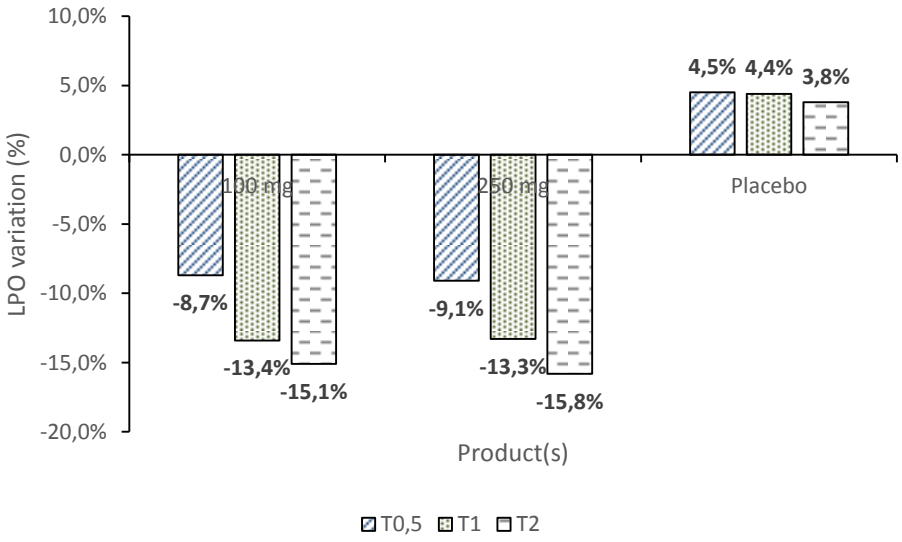
Graph 6b. Variation of UV-induced LPO (24 hours after UV exposure) after product(s) intake.



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3A. CONCLUSION - SHORT TERM TEST

24 hours after UV exposure (T24h) skin redness is significantly ($p<0.05$) increased compared to the basal value (T0h) without any difference ($p>0.05$) among each treatment arm.
Product(s) effect on skin redness, when compared to T24h, is as follows:

- ✓ 100 mg dose: skin redness is statistically significantly ($p<0.05$) decreased starting from T48h;
- ✓ 250 mg dose: skin redness is statistically significantly ($p<0.05$) decreased starting from T25h;
- ✓ Placebo: skin redness is statistically significantly ($p<0.05$) decreased at T72h;

The effect of 100 mg vs 250 mg dose is statistically significant ($p<0.05$) at T25h and T28h. Statistical significance when compared to placebo are as follows:

- ✓ 100 mg dose: skin redness is statistically significantly ($p<0.05$) decreased at T48h;
- ✓ 250 mg dose: skin redness is statistically significantly ($p<0.05$) decreased at T25h, T28h and T48h;

Skin redness at the end of the study (T72h) is not statistically significant ($p>0.05$) when compared to T0h for the 100 and 250 mg treatment arms; while it remains statistically significantly ($p<0.05$) increased in the placebo treatment arm.

Dr. Vincenzo Nobile
International Development Coordinator

3B. CONCLUSION - LONG TERM TEST

Both 100 and 250 mg doses determine a statistically significant ($p<0.05$) improvement of the basal conditions of the skin for all the measured parameters. The effects of 100 and 250 mg doses are not statistically significant ($p>0.05$) when compared each to the other. The effect of 100 and 250 mg doses are statistically significant ($p<0.05$) when compared to the placebo. The variation in the placebo treatment arm are not statistically significant ($p>0.05$).
Results are as follows:

- ✓ 100 and 250 mg doses determine a statistically significant ($p<0.05$) increase of the UV dose necessary to induce a clearly visible erythemal reaction (MED);
- ✓ 100 and 250 mg doses determine a statistically significant ($p<0.05$) increase of the overall skin elasticity (R2: overall elastic recovery of the skin to its basal state) and of the elastic component of the skin (R5: elastic recovery of the skin to its basal state due to its elastic component);
- ✓ 100 and 250 mg doses determine a statistically significant ($p<0.05$) decrease of wrinkle depth;
- ✓ 100 and 250 mg doses determine a statistically significant ($p<0.05$) decrease of the UV-induced lipoperoxidation;

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